The Synthesis of α-Aminonitriles Starting from the Corresponding Amino Acids. I. Use of *o*-Nitrophenylsulfenyl as an N-Protecting Group[1]

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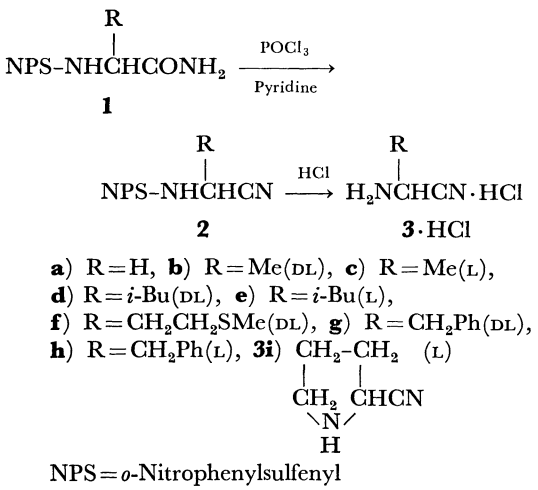
N-*o*-Nitrophenylsulfenylamino acid amides of glycine, alanine (DL and L), leucine (DL and L), methionine (DL), phenylalanine (DL and L), and proline (L) were dehydrated in $POCl_3$–pyridine. The N-protected α-aminonitriles obtained were treated with anhydrous HCl affording the corresponding α-aminonitrile hydrochlorides. The optical purity of the L-α-aminonitriles was well retained.

α-Aminonitriles can be synthesized by reactions of ammonium cyanide with carbonyl compounds, which is known as Zelinsky-Stadnikoff synthesis[2] and which always yields racemic products. It may be more convenient to prepare α-aminonitriles from the corresponding amino acids on a laboratory scale. The purpose of this study is to obtain some racemic and enantiomerically pure α-aminonitriles from the corresponding amino acids, and also to establish a procedure applicable to the enantiomeric analysis of α-aminonitriles.

Several investigators[3–7] have reported the preparation of N-protected α-aminonitriles by dehydrating the corresponding amino acid amides. They employed benzyloxycarbonyl, phthaloyl, or acetyl as an N-protecting group. These N-protecting groups have not been removed. Recently, Ojima *et al.*[8] have reported the asymmetric synthesis of some α-aminonitriles *via* cyanosilylation of the Schiff bases.

Alanyl-α-aminopropiononitriles have been obtained as hydrochlorides by the dehydration of the corresponding *N*-*o*-nitrophenylsulfenyl(NPS)-dipeptide amides, followed by treatment with anhydrous HCl.[9] The present paper is concerned with the preparation of α-aminonitriles, which includes the dehydration of NPS-amino acid amides and the subsequent removal of the N-protecting groups.

As model compounds, NPS derivatives[10] of α-aminonitriles (aminoacetonitrile and DL-α-aminoglutaronitrile) were prepared. Their NPS groups were easily removed by treatment with anhydrous HCl, and ion-exchange chromatography of each α-aminonitrile obtained gave a single peak. On the basis of this work, the nine α-aminonitrile hydrochlorides were synthesized as shown in Scheme 1.



Scheme 1.

The methyl esters of NPS-glycine and alanine (DL and L) were easily converted into the corresponding amides (**1a**—**c**) by ammonolysis at room temperature for several days in methanol saturated with NH_3. The other NPS-amino acid amides (**1d**—**i**) were not obtained by ammonolysis of the corresponding NPS-amino acid methyl esters but were prepared by the reaction of the amino acid amides with *o*-nitrobenzensulfenyl chloride. The NPS-amino acid amides (**1a**—**i**) were dehydrated in cold $POCl_3$–pyridine according to the method of Liberek *et al.*[4] to afford the corresponding nitriles (**2a**—**i**), among which the N-protected nitrile derivatives of glycine, alanine (DL and L), and DL-leucine (**2a**—**d**) were obtained as crystalline products and those of the other amino acids (**2e**—**i**) as oily products. When the NPS-α-aminonitriles (**2a**—**i**) dissolved in ethyl acetate or diethyl ether were treated with anhydrous HCl, the α-aminonitrile hydrochlorides (**3a**—**i**·HCl) shown in Table 1, were obtained. The yields of the products based on the corresponding NPS-amino acid amides (**1a**—**i**) were poor (10—40%). The IR spectra of the products had absorption bands at 2250—2260 cm^{-1} corresponding to the C≡N stretching vibration.

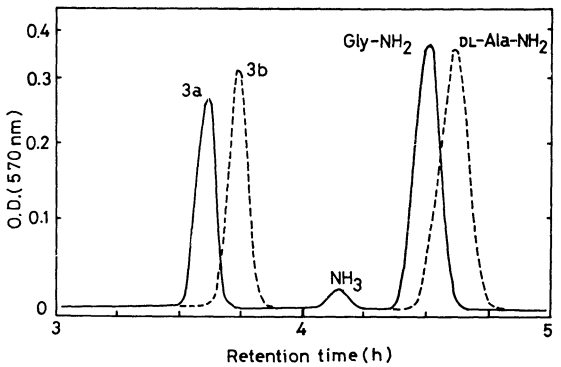


Fig. 1. Ion-exchange chromatograms of α-aminonitriles.
——: **3a**(0.305 μmol)+Gly-NH_2(0.229 μmol).
----: **3b**(0.118 μmol)+DL-Ala-NH_2(0.204 μmol).
Column: 0.25φ×50 cm. Thirty min after the elution began, the eluent was changed from a pH 3.25 buffer to a pH 5.28 buffer.

Table 1. α-Aminonitrile hydrochlorides

α-Amino-nitrile·HCl	Molecular formura	Mp (°C) (dec)	Yield (%)[a]	$\nu_{C\equiv N}$[b] (cm^{-1})	$[\alpha]_D^{22}$ (deg)	Found (Calcd) (%) C	H	N
3a	$C_2H_4N_2$·HCl	164—166[c]	9	2260		26.30 (25.96)	5.47 5.45	30.21 30.28)
3b	$C_3H_6N_2$·HCl	135—137[d]	17	2260		34.16 (33.82)	6.62 6.62	26.28 26.29)
3c	$C_3H_6N_2$·HCl	156—158	13	2260	−15.3 (*c* 0.708, H_2O)	33.77 (33.82)	6.69 6.62	26.37 26.29)
3d	$C_6H_{12}N_2$·HCl	203—208[e]	9	2260		48.33 (48.49)	8.66 8.82	18.60 18.85)
3e	$C_6H_{12}N_2$·HCl	173—176	13	2260	+16.8 (*c* 0.80, H_2O)	48.28 (48.49)	8.86 8.82	18.90 18.85)
3f	$C_5H_{10}N_2S$·HCl	112—115	40	2260		35.91 (36.03)	6.59 6.65	17.15 16.81)
3g	$C_9H_{10}N_2$·HCl	166—168	35	2250		59.09 (59.18)	6.21 6.07	15.26 15.34)
3h	$C_9H_{10}N_2$·HCl	176—178	30	2250	+5.8 (*c* 0.79, H_2O)	58.88 (59.18)	6.14 6.07	15.29 15.34)
3i[f]	$C_5H_8N_2$·HCl	128—130	34	2250	−25.5 (*c* 0.80, H_2O)	44.86 (45.29)	6.94 6.84	20.05 21.13)

a) Calculated on the basis of **1**. b) IR spectra were measured in KBr pellets. c) Lit, mp (dec) 165 °C: Ref. 16. d) Lit, mp (dec) 125—128 °C: Ref. 3 and lit, mp 115—117 °C: Ref. 17. e) Lit, mp (dec) 175—180 °C: Ref. 18. f) Hygroscopic and contaminated with impurities.

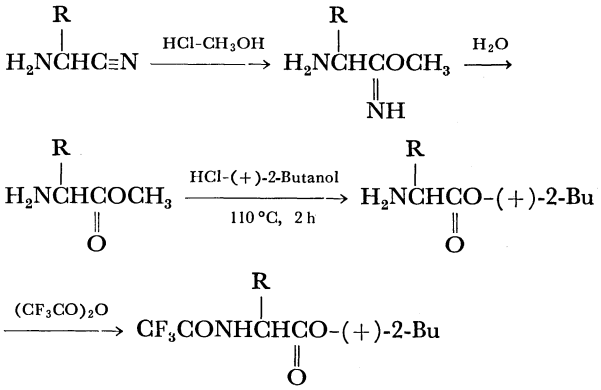
Table 2. Chromatographic data of α-aminonitriles

α-Amino-nitrile	Ion-exchange retention time (h)[a]		Paper[b] R_f[a]
3a	3.6 (4.5)	c)	0.25 (0.29)
3b, **3c**	3.7 (4.6)	c)	0.55 (0.49)
3d, **3e**	4.0 (3.7)	d)	0.86 (0.69)
3f	2.5 (4.4)	d)	0.70 (0.63)
3g, **3h**	5.2 (6.9)[e]	d)	0.80 (0.63)
3i	1.8 (2.4)	d)	0.43 (0.53)

a) Values in parentheses are those of the corresponding amino acid amides. b) Carried out with Toyo filter paper No. 50 using a *n*-BuOH–AcOH–H_2O (4 : 1 : 2 v/v) solvent system. c) 0.25ϕ × 50 cm column. Thirty min after the elution began, the eluent was changed from a pH 3.25 buffer to a pH 5.28 buffer. d) 0.25ϕ × 15 cm column, eluent: pH 5.28 buffer. e) Eluent: pH 5.28 buffer–*t*-BuOH (8 : 2 v/v).

The results of ion-exchange chromatography of the α-aminonitriles (**3a**—**i**) are summarized in Table 2. Only the small peaks of NH_3 were observed in the chromatograms, in addition to the α-aminonitriles. These α-aminonitriles were eluted more rapidly than the corresponding amides with the exception of the α-aminoisocapronitriles (**3d** and **e**), which were not separated from leucine amide when a 0.25ϕ × 15 cm column and a pH 5.28 buffer were used. The chromatograms presented in Fig. 1 show that the chromatographic method is available for quantitative analysis of the α-aminonitriles. Aminoacetonitrile (**3a**) and DL-α-aminopropiononitrile (**3b**) were identified by comparing their retention times on the chromatograms with those of authentic samples. The results of paper chromatography of the α-aminonitriles (**3a**—**i**) are also summarized in Table 2. In this case, **3d** and **3e** were separated from the corresponding amides.

Configuration retention for the L-α-aminonitriles (**3c**, **e**, **h**, and **i**) was confirmed using a gas chromatographic method.[11] These compounds were transformed into the corresponding *N*-trifluoroacetyl(TFA)-amino acid (+)-2-butyl esters as shown in Scheme 2.



Scheme 2.

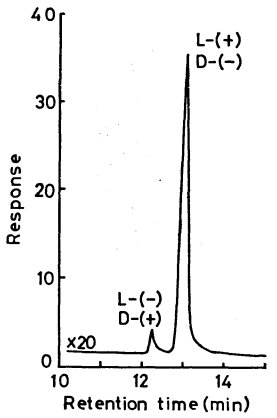


Fig. 2. Gas chromatogram of *N*-TFA-analine (+)-2-butyl ester derived from **3c**.

TABLE 3. A COMPARISON OF THE GAS CHROMATOGRAMS OF *N*-TFA-AMINO ACID (+)-2-BUTYL ESTERS DERIVED FROM BOTH L-AMINO ACIDS AND L-α-AMINONITRILES

Derivative	Retention time (min)		Fraction of peak height[c]	
	I[a]	II[b]	Derivative of L-amino acid	Derivative of L-α-aminonitrile
Ala	12.2	13.0	0.93	0.93 (**3c**)
Leu	21.8	22.8	0.93	0.93 (**3e**)
Phe	64.0	64.8	0.92	0.94 (**3h**)
Pro	44.4	45.2	0.94	0.93 (**3i**)

a) D-(+), L-(−) Diastereomer, b) L-(+), D-(−) Diasteromer. c) Peak height of II/sum of peak heights of I and II.

Each of the *N*-TFA-amino acid (+)-2-butyl esters was analyzed by gas chromatography on a capillary column coated with LB 550X. The *N*-TFA-L-amino acid (+)-2-butyl esters derived from L-amino acids (alanine, leucine, phenylalanine, and proline) were also analyzed. The chromatogram of the sample derived from **3c** is presented in Fig. 2. The sample from L-alanine also gave a similar chromatogram, which shows that the (+)-2-butanol[12] used in this work contains a small amount of the (−)-isomer. The gas chromatographic data are summarized in Table 3, which shows that the fraction of observed peak heights of the sample for each L-α-aminonitrile is approximately equal to that of the sample for the corresponding standard L-amino acid. These results indicate that the enantiomeric purity of the L-α-aminonitriles (**3c**, **e**, **h**, and **i**) is well retained and also that the procedure described in Scheme 2 is available for the enantiomeric analysis of α-aminonitriles.

The poor yields of **2a**—**d** may be due to the unfavorable elimination of the NPS group during the dehydration of the corresponding amides (**1a**—**d**) in $POCl_3$–pyridine. Although the overall yields of **3a**—**i** are also poor, the method described in Scheme 1 may be useful for preparing racemic and enantiomeric α-aminonitriles of as high a purity as possible.

Experimental

All the melting points were determined with a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were taken with a Hitachi EPI-G2 spectrometer. The optical rotations were measured with a Yanaco OR-50 polarimeter. Ion-exchange chromatography was carried out with a Sibata amino acid analyzer, AA-600, under the following conditions: column size, $0.25\phi \times 50$ cm or $0.25\phi \times 15$ cm (Aminex A-4); eluent, citrate buffer of pH 3.25 (0.2 M Na) and pH 5.28 (0.35 M Na); flow rate, 6 ml/h; jacket temperature, 30 °C. Gas chromatography was carried out with a Hitachi gas chromatograph 063 provided with a flame ionization detector; column, 0.01ϕ in $\times 150$ ft stainless steel capillary column coated with LB 550X (Perkin Elmer); temperature 100—170 °C, 1 °C/min; carrier gas He, 6 ml/min. Amino acid methyl ester hydrochlorides were prepared by the thionyl chloride method.[13] *o*-Nitrobenzenesulfenyl chloride was prepared from bis(*o*-nitrophenyl) disulfide and chlorine.[14] Amino acid amides were prepared according to the method of Yang and Rising.[15]

NPS-α-aminonitriles. The neutral sulfate of the α-aminonitrile (aminoacetonitrile or DL-α-aminoglutaronitrile) (0.04 mol) was dissolved in a mixture of 2 M aq NaOH (20 ml) and dioxane (25 ml). Into the solution, *o*-nitrobenzenesulfenyl chloride (0.04 mol) was added in small portions as 2 M aq NaOH (24 ml) were added dropwise, with vigorous stirring during a period of 20 min. The reaction mixture was filtered and diluted with water (400 ml). A yellow product precipitated out, which was recrystallized from ethyl acetate–petroleum ether.

NPS-aminoacetonitrile: Yield, 80%; mp 112—114 °C. Found: C, 46.12; H, 3.50; N, 20.09%. Calcd for $C_8H_7N_3O_2S$: C, 45.93; H, 3.37; N, 20.08%.

NPS-DL-α-aminoglutaronitrile: Yield, 73%; mp 100—102 °C. Found: C, 50.50; H, 3.70; N, 21.21%. Calcd for $C_{11}H_{10}N_4O_2S$: C, 50.37; H, 3.84; N, 21.36%.

Removal of the NPS from the Model NPS-α-aminonitriles. When 5 ml of diethyl ether including anhydrous HCl (3 M) was added to the solution of the NPS-α-aminonitrile (aminoacetonitrile or DL-α-aminoglutaronitrile) (5 mmol) in a minimum amount of ethyl acetate, the α-aminonitrile hydrochloride precipitated out. The precipitates collected were washed with diethyl ether and dried in a vacuum without further purification. The recovery yields of aminoacetonitrile and DL-α-aminoglutaronitrile were 58 and 69%, respectively. The results of ion-exchange chromatography of the products showed that the samples included no impurities such as the corresponding amino acids or amino acid amides.

NPS-amino Acid Methyl Esters. These compounds were prepared according to the method of Zervas *et al.*[10]

NPS-DL-alanine Methyl Ester: Yield, 60% mp 79—81 °C. Found: C, 46.94; H, 4.65; N, 10.76%. Calcd for $C_{10}H_{12}N_2O_4S$: C, 46.87; H, 4.72; N, 10.93%.

Ammonolysis of NPS-amino Acid Methyl Esters. The NPS-amino acid methyl esters of glycine, DL-alanine, and L-alanine were dissolved in small amounts of ethanol and then large excess amounts of methanol saturated with NH_3 were added. The solutions were allowed to stand at room temperature for 2—3 d. The crystals precipitated were collected and recrystallized from hot ethanol. The experimental results for the NPS-amino acid amides (**1a**—**c**) are shown in Table 4.

*NPS-amino Acid Amides (**1d**—**i**).* These compounds were prepared by reactions of the amino acid amides with *o*-nitrobenzenesulfenyl chloride in chloroform in the presence of triethylamine and recrystallized from hot ethanol. In the case of **1d**, the reaction was carried out in chloroform–ethanol (1 : 1 v/v). The experimental results for the NPS-amino acid amides (**1d**—**i**) are shown in Table 4.

*NPS-α-aminonitriles (**2a**—**i**).* An NPS-amino acid amide dissolved in a minimum amount of pyridine or pyridine–dimethylformamide (2 : 1 v/v) was cooled on an ice salt bath below −5 °C and a slight excess of $POCl_3$ was added dropwise to the solution. After the mixture had been allowed to stand at room temperature for 30 min, ice was added to it in order to decompose the excess $POCl_3$ and then the solution was treated with ethyl acetate to extract the product. The extract was washed successively with water, dilute aq HCl, and water, and then dried over anhydrous Na_2SO_4. After evaporation of the ethyl acetate from the extract, the product was separated out as a solid (**2a**—**d**) or an oil (**2e**—**i**) by the addition of petroleum ether. Recrystallization was performed from ethyl acetate–petroleum ether. The crystalline products (**2a**—**d**) obtained are shown in Table 5.

*α-Aminonitrile Hydrochlorides (**3a**—**i**·HCl).* Into a solution of an NPS-α-aminonitrile in a minimum amount of ethyl acetate or diethyl ether were added two equivalents of anhydrous HCl in diethyl ether to afford the α-aminonitrile

Table 4. NPS-amino acid amides

NPS-amino acid amide[a]	Molecular formula	Mp (°C)	Yield (%)	$[\alpha]_D^{30}$ (deg)	Found (Calcd) (%) C	H	N
1a	$C_8H_9N_3O_3S$	166—168	83		42.40 (42.28	4.03 3.99	18.27 18.49)
1b	$C_9H_{11}N_3O_3S$	182—184	78		45.02 (44.80	4.65 4.60	17.27 17.42)
1c	$C_9H_{11}N_3O_3S$	199—201	53	−49.0 (*c* 1.0, DMF)	45.02 (44.80	4.72 4.60	17.62 17.42)
1d	$C_{12}H_{17}N_3O_3S$	206—210	60		51.08 (50.87	6.09 6.05	14.56 14.83)
1e	$C_{12}H_{17}N_3O_3S$	95—98	32	−24.8 (*c* 1.0, DMF)	50.70 (50.87	6.16 6.05	14.57 14.83)
1f	$C_{11}H_{15}N_3O_3S_2$	135—139	41		43.69 (43.84	5.08 5.02	14.04 13.94)
1g	$C_{15}H_{15}N_3O_3S$	122—124	57		56.46 (56.77	4.90 4.76	13.16 13.24)
1h	$C_{15}H_{15}N_3O_3S$	120—122	50	+36.6 (*c* 1.0, DMF)	57.04 (56.77	4.82 4.76	13.10 13.24)
1i	$C_{11}H_{13}N_3O_3S$	192—194	45	−23.4 (*c* 1.0, DMF)	49.17 (49.43	4.89 4.90	15.58 15.72)

a) **1a—c** were obtained by ammonolysis of the NPS-amino acid methyl esters. The others were obtained by reactions of the amino acid amides with *o*-nitrobenzensulfenyl chloride.

Table 5. NPS-α-aminonitriles

NPS-α-amino-nitrile	Molecular formula	Mp (°C)	Yield (%)	$\nu_{C\equiv N}$[a] (cm^{-1})	$[\alpha]_D^{30}$ (deg)	Found (Calcd) (%) C	H	N
2a	$C_8H_7N_3O_2S$	114—116	22	2240		45.88 (45.93	3.38 3.37	20.04 20.08)
2b	$C_9H_9N_3O_2S$	98—100	30	2230		48.14 (48.42	3.77 4.06	18.50 18.82)
2c	$C_9H_9N_3O_2S$	108—110	32	2230	−28.6 (*c* 1.0, DMF)	48.40 (48.42	4.11 4.06	18.48 18.82)
2d	$C_{12}H_{15}N_3O_2S$	53—54	37	2230		54.26 (54.32	5.72 5.70	15.78 15.84)

a) IR spectra were measured in KBr pellets.

hydrochloride. After a large amount of diethyl ether had been added to it, the precipitated crystals were collected and recrystallized from ethanol–diethyl ether.

Derivation of L-α-*Aminonitriles for Gas Chromatographic Analysis.* About 10 mg of each L-α-aminonitrile hydrochloride were dissolved in 9 M HCl in methanol (10 ml) and then water (0.05 ml) was added to the solution. After 2 d, the solution was evaporated to dryness to remove the excess methanol and the residue (amino acid methyl ester hydrochloride) was heated to 100—110 °C for 2 h in (+)-2-butanol (1 ml) saturated with anhydrous HCl. After transesterification, the solution was evaporated to dryness in a vacuum to remove the excess (+)-2-butanol. Trifluoroacetic anhydride (1 ml) was added to the residue and then a clear solution was obtained. Methylene dichloride (5 ml) was added to the solution and it was allowed to stand overnight at room temperature. After trifluoroacetylation, the solution was evaporated to dryness carefully and the residue was taken up in nitromethane for direct injection into the gas chromatographic column.

Preparation of N-*TFA*-DL- *and* L-*amino Acid (+)-2-Butyl Esters.* About 10 mg of the amino acid were suspended in (+)-2-butanol (1 ml) saturated with anhydrous HCl. The suspension was heated to 100—110 °C for 3 h and then a clear solution was obtained. After esterification, the solution was evaporated to dryness in a vacuum and trifluoroacetylation was carried out in a manner similar to that described above.

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